LOW TEMPERATURE BLOCKAGE OF MOLTING IN UCA PUGNAX

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The fiddler crabs, members of the genus *Uca,* have been the favorite animals for the experimental investigation of molting in brachyurans by American comparative endocrinologists. The facility in collecting and holding these sand and mud flat burrowers and the simplicity of inducing proecdysis by bilateral eyestalk removal have resulted in use of these crabs and this technique by many investigators during the past two decades. Casual note has been made of the variation in proecdysis duration observed, some of which has been attributed to differences in ambient temperatures. Presumably because of limited facilities, no systematic study has been made of the effect of temperature on either proecdysis duration or proecdysis initiation.

In a preliminary observation incidental to a previously reported study of the endocrinological basis of molting (Passano, 1953) a group of 20 male *Uca pugnax* had both eyestalks removed in early winter and were then kept for 35 days in individual bowls at room temperatures diurnally fluctuating between 14° and 21° C. There was a high mortality rate, but of the 8 crabs surviving, none molted. When put in a 27° constant temperature room, three crabs molted after 3, 7, and 8 days. Since eyestalkless *U. pugnax* kept at 23.5° C. showed an average proecdysis period of 19.3 days (Passano, 1953), it appeared as though a 5° reduction in temperature might block or markedly delay a forced ecdysis initiated by eyestalk removal (*i.e.,* by extirpation of the X-organ sinus gland complex).

The experiment reported here has two parts: 1) exposure to a constant test temperature for 23 days, following elimination of the molt-inhibiting hormone (MIH) by bilateral eyestalk removal, and 2) a post-treatment period at the optimum temperature for induced proecdysis, to measure the extent of the previous temperature-induced proecdysis block.

Materials and Methods

Approximately 600 male marsh fiddler crabs, *Uca pugnax* (S. I. Smith), were collected from a salt marsh near New Haven, Connecticut, in late April (water temperature 12° C., air temperature 8°–15° C.) and acclimatized to 15° for one week. Four hundred animals of uniform size were then selected, discarding any whose major chela was missing or undersized, or which had autotomized more than one walking leg, so that the selected crabs had approximately the same blood volume. One eyestalk was removed by cutting across the arthrodial membrane at the base of the eyestalk with fine scissors and each animal was forced to autotomize one of its walking legs, usually the fourth leg on the side opposite to the major chela. The following day the other eyestalk was removed in a like manner. Twelve hours later the surviving eyestalkless animals were divided into ten groups

of 38 crabs each, hereafter called Groups A, B, C . . . J, with the use of a table of random numbers. Subsequent measurement showed that these groups were homogeneous in size distribution with a standard length mean, and standard deviation of the mean, of 18.7 ± 1.2 mm. (Table I). Each animal was put in an individual covered glass dish (or "fingerbowl") with sufficient water (1:1 Long Island Sound Water:tap water; salinity 14‰) to cover the maxillipeds. The water was changed on the sixth, twentieth and thirty-third days after eyestalk removal. Each group was placed in a separate temperature-controlled room (Table I) and kept there for the temperature treatment period of 23 days. Those rooms closest to ambient temperatures, 20° and 22°, showed the largest fluctuations in air temperature, but these rarely exceeded 1° and were rapid and cyclic. A control group of 19 crabs was placed at the warmest temperature after forced

TABLE I

The effect of exposure to different temperatures on ecdysis by eyestalkless Uca

Group	Number (after initial mortality)	Size (standard length, mm.)	Temperature treatment (23 days)			Post-treatment (20 days at 29°)		Total	
			Temp. ° C.	% molt	% remaining	% molt	% remaining	% molt	Mean proecdysis duration and S.D.
A	37	18.7	13.1	0	92	46	14	46	37.4± .85
B	37	18.6	15.3	0	86	43	26	43	36.8± .79
C	37	18.5	16.3	0	97	54	19	54	35.7±1.00
D	37	18.6	18.2	0	97	59	16	59	33.4± .97
E	38	18.7	20.0	0	100	58	13	58	31.8±1.05
F	36	18.5	22.2	25	72	31	8	56	24.8±1.15
G	37	18.8	24.4	73	16	11	0	84	19.3± .61
H	35	18.5	26.7	76	17	6	0	81	17.4± .63
I	37	18.5	29.4	89	5	0	0	89	14.8± .73
J	37	18.8	32.2	87	0	0	0	87	14.4± .49
Controls	19	19.0	32.2	0	53	0	11	0	—
	387	18.7±1.2							

autotomy of one walking leg, but as expected, none molted nor showed signs of entering proecdysis. Since the experimental animals were blinded, no regular photoperiods were given. The crabs were not fed, inasmuch as it was impossible to insure equal uptake in the different temperature groups or by individual crabs at the same temperature.

Mortality during the temperature treatment period of the experiment was light. Deaths during the first week (3.2%) are considered to be due to operational mortality and are not included in the tabulated results.

Although some animals successfully completed ecdysis following the experimental treatment, the largest number died during ecdysis or were unable to withdraw all of their appendages. Any crabs dying in Stage E (Drach, 1939) were scored as molted, while those few dying in Stage D_4 were scored as having molted the following day. No animals were kept after ecdysis.

After 23 days, those crabs remaining were examined for evidence of proecdysis initiation. The regeneration of the previously autotomized fourth walking leg was used as an indicator (Bliss, 1956; Jyssum and Passano, 1957). The animals were then slowly equilibrated to 29.4° and kept there for a further 20 days to determine what effect, if any, the initial temperature treatment had had on proecdysis initiation. When the experiment was ended, 43 days after removal of the second eyestalk, 8.5% of the animals remained.

Results

Following elimination of the MIH by bilateral eyestalk removal, a total of 242 of the 368 animals (66%) eventually reached or completed ecdysis. None of the control crabs molted. Table I and Figures 1 and 2 show the differential effect of

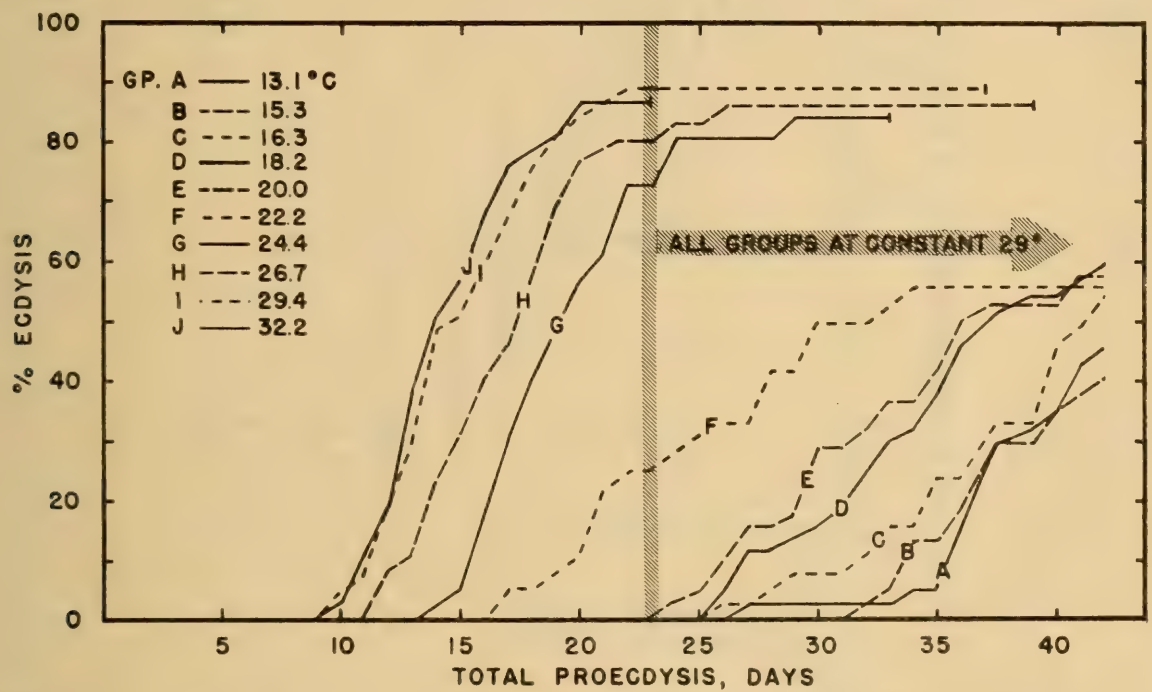

Figure 1. Cumulative percentages of *Uca* molting in each experimental group following eyestalk extirpation.

temperature on average proecdysis duration, the per cent completing proecdysis and the uniformity of response within each group. The low values of the standard errors of the mean for the proecdysis duration of each group are noteworthy.

1. Low temperature treatment, Groups A, B, and C

Exposure to constant low temperature in the range from 13.1° to 15.3° C. blocks proecdysis initiation almost completely, in spite of elimination of the MIH. With a single exception in the group kept at the lowest temperature, none of the Group A or Group B animals initiated discernible proecdysis during the temperature treatment period. Only 14% of these crabs showed basal limb bud (Bliss, 1956) regeneration of their autotomized pereiopod, itself a proecdysis-independent process (Jyssum and Passano, 1957). Again with the single exception already

noted, none reached the ecdysis stage, Stage E (Drach, 1939), in less than 9 days after being placed at 29.4° (Fig. 1). The mean proecdysis duration in the post-treatment warm temperature for Groups A and B combined was 14.1 days with a standard error of the mean of ± 0.48 days. This is not significantly different from the value for Group I crabs which were put directly at 29.4° after eyestalk removal (Fig. 2, shaded bar), although it is shorter.

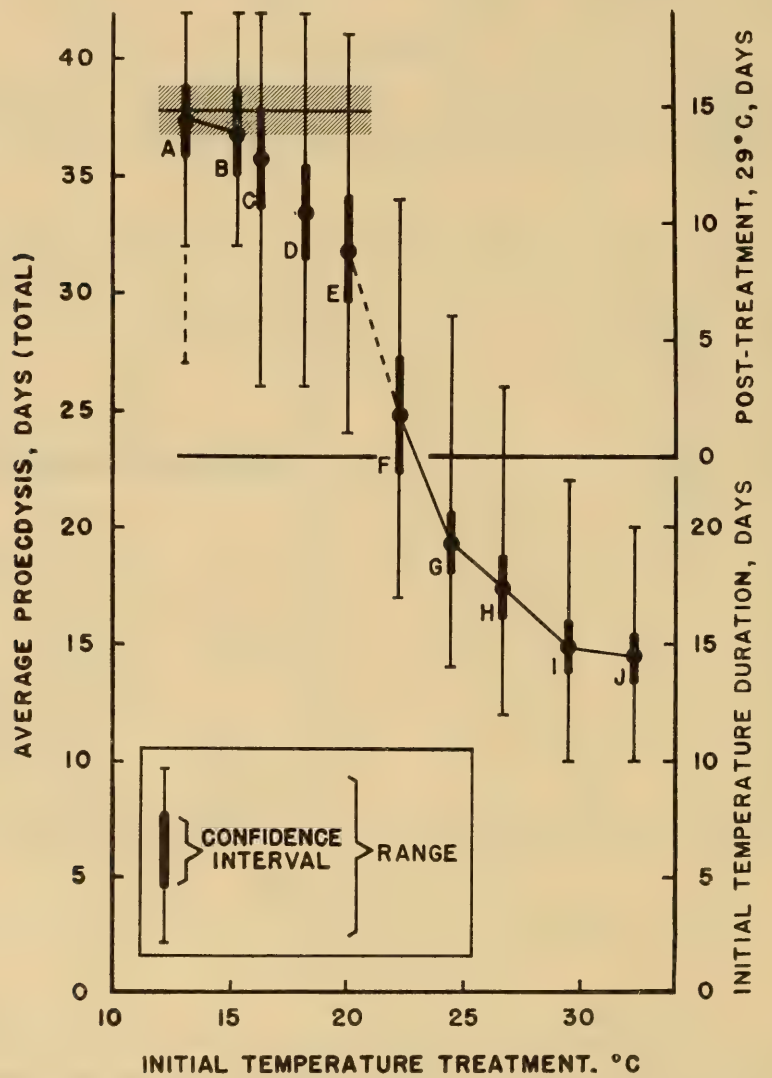

FIGURE 2. The effect of various initial temperature treatments on the proecdysis duration of eyestalkless *Uca*. The shaded horizontal bar represents the confidence interval (13.7–15.9 days) for animals put directly into 29.4° C., transposed to the upper, post-treatment, portion of the graph.

Group A's exceptional animal molted on the fourth day of post-treatment warm temperature. It showed no limb regeneration whatsoever. Since it is probable that this individual had already commenced proecdysis prior to the beginning of the experiment, the increase in the extremes of proecdysis duration caused by this aberrant animal is shown by a dashed line in Figure 2.

Group C, kept at 16.3° for the temperature treatment period, also shows a

post-treatment proecdysis duration, 12.7 ± 1.0 days, insignificantly different from that of Groups A and B. However, half the crabs showed some basal limb bud regeneration after 23 days at 16.3° and one crab, which molted six days later after being raised to 29.4°, had a "premolt limb bud" (Jyssum and Passano, 1957) at that time. The other three animals which molted prior to the tenth post-treatment day again showed no limb regeneration at all.

2. High temperature treatment, Groups G, H, I and J

There was no discernible proecdysis blockage in the groups of animals kept at 24.4° or above (Figs. 1, 2). The two groups kept at the highest temperatures, J (32.2°) and I (29.4°), had average proecdysis durations of 14.4 ± 0.49 days and 14.8 ± 0.73 days, respectively, the slight difference being without statistical validity. The somewhat greater standard deviation of Group I is due to a slight skew of the distribution curve towards the longer proecdysis (Fig. 1). In neither case did any molting occur prior to 10 days, nor 22 days after, removal of the second eyestalk. Molting occurred in 88% of Groups I and J, and of these 94% regenerated the autotomized pereiopod.

Groups H (26.7°) and G (24.4°) also showed a nearly uniform molting response but here proecdysis duration was significantly increased ($P < 0.01\%$; $P < 0.04\%$). As in the groups kept at the highest temperatures, nearly all regenerated their autotomized walking leg.

3. Intermediate temperature, Groups D, E, and F

None of the crabs kept at 18.2° (Group D) or 20.0° (Group E) reached ecdysis during the temperature treatment period, but examination of the regenerating limb buds showed that at least 50% were in proecdysis. During the post-treatment period at 29.4°, approximately half of those crabs which eventually did reach ecdysis had done so before the temperature-blocked animals of Groups A, B and C began to molt (Fig. 1). Nearly all of the Group F molts (22.2°) had molted by this time.

The largest standard deviation from mean proecdysis duration occurred within these intermediate temperature groups, in Group F. Many of those animals in which proecdysis appeared to be blocked failed to survive the 20-day post-treatment period at 29.5° (Table I).

Discussion and Conclusions

Molting after bilateral eyestalk extirpation is due to elimination of molt-inhibiting hormone (MIH) of the medulla terminalis ganglionic X-organ (Bliss, 1953; Passano, 1953) and consequent formation and release of molting hormone (MH) by the Y-organ (Echalier, 1959). MH then causes the sequence of morphogenic and biochemical events collectively termed proecdysis, ecdysis and postecdysis.

The results reported here show that proecdysis duration is strongly temperature-dependent. In *Uca pugnax* a temperature between 29° and 32° gives the shortest average proecdysis duration. Temperatures above this may be deleterious to this

crab, since most of the normal control animals failed to survive the 42 days of this experiment, but it is equally plausible that these animals starved to death. Temperatures somewhat below 29° still permit molting but the proecdysis period is progressively lengthened, doubling in duration when the temperature falls from 30° to 20°.

Temperatures of 15° or below effectively block proecdysis. A comparison between either Group A or Group B and Group I shows that mean proecdysis duration at 29° is nearly the same whether or not the eyestalkless animals had first been kept at 15° for three weeks (Fig. 2). No appreciable proecdysis occurs during this period in these groups. The low temperature treatment did reduce the total percentage molting to one-half its original value (Table I). This is presumably due to the continuous depletion of organic reserves during the three weeks of low temperature treatment starvation. If the experimental animals had been fed, this decrease in the percentage of the group reaching ecdysis would probably have not occurred.

At some temperature between 15° and 22° proecdysis may either fail to commence, or else may be initiated but proceed slowly, after eyestalk removal. The lengthened mean proecdysis durations of the intermediate temperature groups, D (18°) and E (20°), are due in part to proecdysis blockage in some of the crabs. After three weeks at the treatment temperatures, 42% of the 18° animals and 39% of the 20° animals showed no signs of proecdysis initiation. The remaining crabs had initiated proecdysis but its duration was lengthened by the "low" temperature. It is thus to be expected that these intermediate temperature groups are the most heterogeneous in their response of all the temperature groups tested (Fig. 2).

It is scarcely surprising to find an inverse correlation between temperature and proecdysis duration. It is unusual to find that a temperature of 15° or even higher can block completely an animal's growth, even though this species experiences lower temperatures through much of its life cycle. Since it can survive at these lower temperatures for long periods of time, it seems probable that some key reaction, required in proecdysis initiation, is being blocked. The first half of the proecdysis requires MH, since crabs deprived of their Y-organs are permanently blocked in their intermolt stage (Echalier, 1959). Low temperatures might thus prevent the Y-organs from responding to the elimination of the MIH following eyestalk removal. Alternatively, these glands might form and release into the circulation adequate amounts of MH, but the target tissues might be unable to respond to the MH rise. Yet neither of these explanations accounts for the nearly complete correlation of proecdysis blockage and basal limb bud regeneration blockage found in these experiments. Although the main part of limb regeneration occurs only during proecdysis, so that regenerate size is a good index of proecdysis stage (Bliss, 1956), the initial or basal outgrowth of the regenerating limb is independent of Y-organ MH (Jyssum and Passano, 1957) and, unlike proecdysis initiation, is not photosensitive (Bliss, 1956). Therefore if the temperature-dependent proecdysis blockage found here is blocking Y-organ MH formation or activity, some other temperature-dependent step must be blocking basal limb bud formation in these starved animals.

Although a few of the animals which molted in this experiment failed to show

any limb regeneration, 92% did regenerate the autotomized walking leg. Perhaps temperatures of 15° or below limit some metabolic process common to both proecdysis and basal limb bud formation. One of the initial events in proecdysis is the mobilization of hepatopancreas metabolic reserves (Bliss, 1953; Passano, 1960), noted after eyestalk removal by a rise in oxygen consumption (Edwards, 1950; Bliss, 1953) and a fall in R.Q. (Bliss, 1953). Stored fats are utilized for proecdysis integument growth and mineral mobilization (Renaud, 1949). The basic metabolic requirements of the animal, as well as basal bud regeneration, must require storage depletion as long as animals remain unfed. Perhaps the low temperatures prevent sufficient mobilization of hepatopancreas reserves for basal limb regeneration and proecdysis initiation, as well as basal metabolism. It is possible that such mobilization is normally controlled by an eyestalk hormone in the intact animal at higher temperatures, since eyestalk removal causes a rise in oxygen consumption whether or not the Y-organs are intact (Passano, unpublished). It is not known whether the same rise in O_2 consumption follows bilateral X-organ extirpation in the Y-organless animal; if so, the MIH may be controlling proecdysis initiation by limiting the fat depot mobilization necessary for MH synthesis.

An alternative explanation might be developed from the hypothesis that all crustacean growth processes are controlled in an identical manner (Bliss, 1956). Thus basal limb growth would be under MH control, but this MH would originate in some extra-Y-organ site such as the individual limb bud blastema. It would support this alternative if it were found that Y-organless crabs could be forced into proecdysis by initiating mass basal limb bud regeneration. Multiple regeneration in normal crabs can lead to ecdysis even though environmental conditions are unfavorable (Bliss, 1956), but such treatment might activate their intact Y-organs. If such extra-Y-organ sources of MH occur, then low temperatures could be blocking MH formation or activity, irrespective of the hormone's origin.

Uca (including *U. minax* (Le Conte) and *U. pugilator* (Bosc) in addition to the species used here) occurs on the East Coast of the United States as far north as Cape Cod, but does not commonly occur north of the Cape (Rathbun, 1918). The notable discontinuity at Cape Cod in the littoral fauna is primarily due to the cooler summer water temperatures of the Gulf of Maine, yet the temperature experienced by adult *Uca* must be primarily determined by ambient air temperature rather than water temperatures. Since there is no marked air temperature differential between the littoral areas north and south of Cape Cod, it seems likely that the temperature limit on *Uca* acts on growth of its pelagic larvae rather than on post-larval growth. Perhaps, then, the significance of the proecdysis temperature block found in these experiments is that it reflects an identical limitation of proecdysis initiation occurring in *Uca* larvae, so that larvae hatching in the cooler Gulf of Maine waters are unable to grow because they are unable to molt. Caution must be maintained, however, in attributing species limits to simple temperature effects (Moore, 1958).

Summary

1. A uniform population of male *Uca pugnax* was used in studying the effect of 10 temperatures on proecdysis resulting from eyestalk removal.

2. Proecdysis duration is shortest at 29° to 32° C. and lengthens significantly at lower temperatures.

3. Proecdysis initiation is markedly temperature-sensitive. At 15° or below initiation is completely blocked; at 15° to 20° a substantial proportion of the crabs fail to begin proecdysis.

4. Temperatures which block proecdysis initiation also block basal limb bud regeneration (a molt-independent growth process). It is hypothesized that a metabolic event common to both processes is being blocked.

5. The physiological and ecological significance of the proecdysis temperature block is considered.

LITERATURE CITED

Bliss, D. E., 1953. Endocrine control of metabolism in the land crab, *Gecarcinus lateralis* (Fréminville). I. Differences in the respiratory metabolism of sinusglandless and eyestalkless crabs. *Biol. Bull.*, **104**: 275–296.

Bliss, D. E., 1956. Neurosecretion and the control of growth in a decapod crustacean. *In:* Bertil Hanström, Zoological Papers in Honour of His Sixtyfifth Birthday, November 20th, 1956 (K. G. Wingstrand, ed.), pp. 56–75. Zoological Institute, Lund.

Drach, P., 1939. Mue et cycle d'intermue chez les Crustacés Décapodes. *Ann. Inst. Océanog. (Paris) (N.S.)*, **19**: 103–391.

Echalier, G., 1959. L'organe Y et le déterminisme de la croissance et de la mue chez *Carcinus maenas* (L.), Crustacé decapode. *Ann. Sci. Nat. Zool. et Biol. Animale,* [12], **1**: 1–57.

Edwards, G. A., 1950. The influence of eyestalk removal on the metabolism of the fiddler crab. *Physiol. Comparata et Oecol.*, **2**: 34–50.

Jyssum, S., and L. M. Passano, 1957. Endocrine regulation of preliminary limb regeneration and molting in the crab *Sesarma*. *Anat. Record* **128**: 571–572.

Moore, H. B., 1958. Marine Ecology. John Wiley and Sons, Inc., New York.

Passano, L. M., 1953. Neurosecretory control of molting in crabs by the X-organ sinus gland complex. *Physiol. Comparata et Oecol.*, **3**: 155–189.

Passano, L. M., 1960. Molting and its control. *In:* The Physiology of Crustacea (T. H. Waterman, ed.). Academic Press, Inc., New York. Pp. 473–536.

Rathbun, M. J., 1918. The Grapsoid Crabs of America. *U. S. Natl. Museum Bull.*, **97**: 1–461.

Renaud, L., 1949. Le cycle des réserves organiques chez les Crustacés Décapodes. *Ann. Inst. Océanog. (Paris) (N.S.)*, **24**: 259–357.